with the aim of identifying sites of transmembrane domain interaction. The tryptophan-scanning technique is based on the premise that the large bulky side-chain of tryptophan is tolerated when positioned in a lipid environment but disrupts protein function when inserted at a site of protein interaction. Tryptophan was substituted sequentially for sixteen amino acids within M1 of Shak-B(lethal) and channel function was assayed using the *Xenopus* oocyte expression system. Four sites of transmembrane domain interaction were identified, all positioned along the same helical face of M1. The results suggest that M1 interacts closely with only one other transmembrane helix.

2783-Pos

Temperature dependence of Proton Permeation through a Voltage-Gated Proton Channel in Microglia

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Voltage-gated proton channels are found in many different types of cells, where they facilitate proton movement through the membrane. The mechanism of proton permeation through the channel is an issue of long-term interest, but remains an open question. To address this issue, we examined the temperature-dependence of proton permeation. Under whole-cell recordings rapid temperature changes within a few ms were imposed. This method allowed for the measurement of current amplitudes immediately before and after a temperature jump, from which the ratios of these currents (I_{ratio}) were determined. The use of I_{ratio} for evaluating the temperature dependence minimized the contributions of factors other than permeation. Temperature jumps of various degrees (ΔT; −15 - 15°C) were applied over a wide temperature range (4 - 49°C), and the Q_{10}s for the proton currents were evaluated from the I_{ratio}s. Q_{10} exhibited high temperature dependence, varying from 2.2 at 10°C to 1.3 at 40°C, implying that processes with different temperature dependencies underlie the observed Q_{10} (apparent Q_{10}, Q_{10}^{app}). A novel resistivity pulse method revealed that the access resistance with its low temperature dependence became predominated in high temperature ranges. The Q_{10}^{app} was decomposed into Q_{10} of the channel and of the access resistances. Finally, the Q_{10} for proton permeation through the voltage-gated proton channel itself was calculated and found to vary from 2.8 at 5°C to 2.2 at 45°C as expected for an activation enthalpy of 64 kJ/mol. The thermodynamic features for proton permeation through proton-selective channels would provide an important clue for the permeation mechanism.

2784-Pos

Membrane Topology of S4 of the Mouse Voltage-Gated Proton Channel

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VSOP/Hv1 is a voltage-gated proton channel that contains the voltage sensor domain (VSD) but not pore domain. VSD of VSOP/Hv1 allows protons to permeate as well as sensing voltage. It has been reported that basic amino acids in the fourth transmembrane segment (S4) of voltage-gated ion channels play critical roles in voltage-sensing. Mouse VSOP (mVSOP) has three arginine residues (R1, R2, R3) in a pattern similar to those conserved in other voltage-gated channels. To address the role of S4 in mVSOP, we have reported that the truncated construct (A206sop) just downstream of R2 in the S4 is still ion-conductive (Biophysical Society 53th Annual Meeting, 2009). In this study, we further analysed properties of A206stop. The outward current of A206stop was almost completely blocked by zinc. Visualization of intracellular pH using BCECF (pH-sensitive ratiometric dye) showed that cytoplasm of tsA201 cell was alkalinized under the depolarization condition. Na and K ion do not permeate through A206stop. Gating properties of the proton currents through A206stop were sensitive to either intracellular pH or extracellular pH. However, voltage dependency of A206stop was weaker than that of full-length mVSOP, and the I-V relationship of A206stop was shifted rightward. These results indicate that A206stop retains the basic properties of the voltage-gated proton channel even if it lacks a half of S4. We also carried out two biochemical assays: site-directed cysteine-scanning using accessibility of maleimide-reagent as detected by western blotting (pegylation protection) and in vitro glycosylation assays. Both showed that S4 of A206stop inserts into the membrane and the position of A206 faces intracellular aqueous environments. These findings suggest that the region downstream of the R2 position of S4 of VSOP/Hv1 is not essential for proton selectivity.

2785-Pos

Pharmacological Relevant Amantadine Binding Site is in the Pore of Influenza a Virus M2 Proton Channel

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The influenza A virus M2 protein (A/M2) and the influenza B virus BM2 protein are both homotetrameric pH-activated proton channel that facilitates viral uncoating by acidification the interior of endosomally encapsulated virus. Antiviral drugs amantadine and its derivative rimantadine inhibit A/M2 channel of influenza A virus, but not BM2 channel of influenza B virus. The atomic structure of the pore-transmembrane (TM) domain peptide has been determined by X-ray crystallography (Stouffer et al., Nature 451, 596-599 [2008]) and of a larger M2 peptide by NMR methods (Schnell and Chou, Nature 451, 591-595 [2008]). The crystallographic data shows electron density (at 3.5 Å resolution) in the channel pore, consistent with amantadine blocking the pore of the channel. In contrast, the NMR data show four rimantadine molecules bound on the outside of the helices towards the cytoplasmic side of the membrane. Drug binding includes interactions with residues 40-45 and a hydrogen bond between rimantadine and Asp44. These two distinct drug-binding sites led to two incompatible drug inhibition mechanisms. The cytoplasmic binding site predicts that D44 and R45 to alanine mutations would interfere with rimantadine binding and lead to a drug insensitive channel. However, the D44A channel was found to be sensitive to amantadine when measured by TEVC recordings in oocytes of *Xenopus laevis*, and when the D44 and R45 mutations were introduced into the influenza virus genome. Furthermore, two chimeras containing 5 residues of the A/M2 ectodomain and residues 24-36 of the A/M2 TM domain show 85% amantadine/rimantadine sensitivity and specific activity comparable to wt BM2. These functional data suggest the pharmacological relevant amantadine/rimantadine binding site is in the pore of the M2 channel.

2786-Pos

Ryanodine Receptors Control Cytosolic Calcium Elevation Following Activation of Store-Operated Calcium Entry in Activated but not Resting Human T Lymphocytes

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Previously we have shown that in Jurkat T lymphocytes, the ryanodine (RyR) receptors are activated by store-operated Ca^{2+} entry (SOCE) and that inhibition of RyR significantly reduced elevation in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) following SOCE. Because Jurkat T cells differ from normal human T cells, we explored contribution of RyR into Ca^{2+} signaling in two functional human T lymphocyte subsets: resting and activated. Resting T cells were isolated from the peripheral blood of healthy humans and activated in vitro using anti-CD3 and anti-CD28 antibodies. Assessing the $[Ca^{2+}]_i$ dynamics in activated T cells using fura-2, a Ca^{2+} indicator, revealed that RyR blockers ryanodine (Ry) and dantrolene (Da) significantly reduced Ca^{2+} elevation upon SOCE activation, while increasing Ca^{2+} content within the store, which is consistent with our previous findings in Jurkat T cells. In contrast, in resting T cells neither Ry nor Da affected $[Ca^{2+}]_i$ elevation upon SOCE activation at physiological concentration (2 mM) of extracellular Ca^{2+}. However, the inhibitory effects of RyR blockers were observed in resting T cells in the presence of the elevated extracellular Ca^{2+}concentration (10 mM). Using Mn^{2+} quench of fura 2 fluorescence approach we further explored whether inhibition of $[Ca^{2+}]_i$ elevation in the presence of RyR blockers could be attributed to termination of SOCE due to the Ca^{2+} accumulation within the store. We found that rates of Mn^{2+} quench were identical in the presence and absence of RyR blockers, indicating that within a given timeframe enhanced Ca^{2+} accumulation within the store did not affect SOCE. We conclude that in activated human T cells Ry-sensitive store serves as an intermediate compartment for SOCE and that RyR controls $[Ca^{2+}]_i$ dynamics by regulating $[Ca^{2+}]_i$ release from the store.

2787-Pos

Incorporation of RyR2 and Other Ion Channels into Nanopore Based Planar Lipid Bilayers for Low Noise Single Channel Recordings

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Measurement of ion channel activity at the single molecule level in isolated planar lipid bilayers (PLB) is a critical biophysical tool for understanding the function of such proteins. However, current PLB techniques involving bilayers painted across apertures >100 μm suffer from high noise arising from the capacitance of the bilayer and is severely limited in bandwidth. Therefore, it is